

Applicant : Chappell et al.  
Serial No. :  
Filed :  
Title : SYNTHASES

Art Unit : Unknown  
Examiner : Unknown

Commissioner for Patents  
Washington, D.C. 20231

Prior to examination, please amend the application as follows:

Please insert the following paragraph after the title on page 1 of the application:

This application is a divisional of and claims priority under 35 U.S.C. §120 to U.S.

Please replace the Sequence Listing filed with the application with the enclosed Sequence Listing.

Please cancel claims 1-106 and 108-162 without prejudice to continued prosecution.

Express Mail Label No. EL 734683815US

Date of Deposit June 28, 2001

Signature \_\_\_\_\_

Vince Defante  
Typed or Printed Name of Person Signing Certificate

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Attorney's Docket No.: 07678-025004

The pending claims, including new claims 163-242, are as follows.

107. An isolated synthase having a region with 40% or greater sequence identity to residues 579 to 847 of SEQ ID NO: 44, wherein one or more amino acid residues of said synthase that align with amino acids at positions 584, 587, 606, 609, 610, 688, 713, 714, 715, 716, 719, 753, 757, 831, 834, 835, 839, 841 and 842 of SEQ ID NO: 44 are residues other than the following ordered arrangements of residues:

107. An isolated synthase having a region with 40% or greater sequence identity to residues 579 to 847 of SEQ ID NO: 44, wherein one or more amino acid residues of said synthase that align with amino acids at positions 584, 587, 606, 609, 610, 688, 713, 714, 715, 716, 719, 753, 757, 831, 834, 835, 839, 841 and 842 of SEQ ID NO: 44 are residues other than the following ordered arrangements of residues:

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|  | 1970 | 1971 | 1972 | 1973 | 1974 | 1975 | 1976 | 1977 | 1978 | 1979 | 1980 | 1981 | 1982 | 1983 | 1984 | 1985 | 1986 | 1987 | 1988 | 1989 | 1990 | 1991 | 1992 | 1993 | 1994 | 1995 | 1996 | 1997 | 1998 | 1999 | 2000 | 2001 | 2002 | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 | 2018 | 2019 | 2020 | 2021 | 2022 | 2023 | 2024 | 2025 | 2026 | 2027 | 2028 | 2029 | 2030 | 2031 | 2032 | 2033 | 2034 | 2035 | 2036 | 2037 | 2038 | 2039 | 2040 | 2041 | 2042 | 2043 | 2044 | 2045 | 2046 | 2047 | 2048 | 2049 | 2050 | 2051 | 2052 | 2053 | 2054 | 2055 | 2056 | 2057 | 2058 | 2059 | 2060 | 2061 | 2062 | 2063 | 2064 | 2065 | 2066 | 2067 | 2068 | 2069 | 2070 | 2071 | 2072 | 2073 | 2074 | 2075 | 2076 | 2077 | 2078 | 2079 | 2080 | 2081 | 2082 | 2083 | 2084 | 2085 | 2086 | 2087 | 2088 | 2089 | 2090 | 2091 | 2092 | 2093 | 2094 | 2095 | 2096 | 2097 | 2098 | 2099 | 2100 | 2101 | 2102 | 2103 | 2104 | 2105 | 2106 | 2107 | 2108 | 2109 | 2110 | 2111 | 2112 | 2113 | 2114 | 2115 | 2116 | 2117 | 2118 | 2119 | 2120 | 2121 | 2122 | 2123 | 2124 | 2125 | 2126 | 2127 | 2128 | 2129 | 2130 | 2131 | 2132 | 2133 | 2134 | 2135 | 2136 | 2137 | 2138 | 2139 | 2140 | 2141 | 2142 | 2143 | 2144 | 2145 | 2146 | 2147 | 2148 | 2149 | 2150 | 2151 | 2152 | 2153 | 2154 | 2155 | 2156 | 2157 | 2158 | 2159 | 2160 | 2161 | 2162 | 2163 | 2164 | 2165 | 2166 | 2167 | 2168 | 2169 | 2170 | 2171 | 2172 | 2173 | 2174 | 2175 | 2176 | 2177 | 2178 | 2179 | 2180 | 2181 | 2182 | 2183 | 2184 | 2185 | 2186 | 2187 | 2188 | 2189 | 2190 | 2191 | 2192 | 2193 | 2194 | 2195 | 2196 | 2197 | 2198 | 2199 | 2200 | 2201 | 2202 | 2203 | 2204 | 2205 | 2206 | 2207 | 2208 | 2209 | 2210 | 2211 | 2212 | 2213 | 2214 | 2215 | 2216 | 2217 | 2218 | 2219 | 2220 | 2221 | 2222 | 2223 | 2224 | 2225 | 2226 | 2227 | 2228 | 2229 | 2230 | 2231 | 2232 | 2233 | 2234 | 2235 | 2236 | 2237 | 2238 | 2239 | 2240 | 2241 | 2242 | 2243 | 2244 | 2245 | 2246 | 2247 | 2248 | 2249 | 2250 | 2251 | 2252 | 2253 | 2254 | 2255 | 2256 | 2257 | 2258 | 2259 | 2260 | 2261 | 2262 | 2263 | 2264 | 2265 | 2266 | 2267 | 2268 | 2269 | 2270 | 2271 | 2272 | 2273 | 2274 | 2275 | 2276 | 2277 | 2278 | 2279 | 2280 | 2281 | 2282 | 2283 | 2284 | 2285 | 2286 | 2287 | 2288 | 2289 | 2290 | 2291 | 2292 | 2293 | 2294 | 2295 | 2296 | 2297 | 2298 | 2299 | 2300 | 2301 | 2302 | 2303 | 2304 | 2305 | 2306 | 2307 | 2308 | 2309 | 2310 | 2311 | 2312 | 2313 | 2314 | 2315 | 2316 | 2317 | 2318 | 2319 | 2320 | 2321 | 2322 | 2323 | 2324 | 2325 | 2326 | 2327 | 2328 | 2329 | 2330 | 2331 | 2332 | 2333 | 2334 | 2335 | 2336 | 2337 | 2338 | 2339 | 2340 | 2341 | 2342 | 2343 | 2344 | 2345 | 2346 | 2347 | 2348 | 2349 | 2350 | 2351 | 2352 | 2353 | 2354 | 2355 | 2356 | 2357 | 2358 | 2359 | 2360 | 2361 | 2362 | 2363 | 2364 | 2365 | 2366 | 2367 | 2368 | 2369 | 2370 | 2371 | 2372 | 2373 | 2374 | 2375 | 2376 | 2377 | 2378 | 2379 | 2380 | 2381 | 2382 | 2383 | 2384 | 2385 | 2386 | 2387 | 2388 | 2389 | 2390 | 2391 | 2392 | 2393 | 2394 | 2395 | 2396 | 2397 | 2398 | 2399 | 2400 | 2401 | 2402 | 2403 | 2404 | 2405 | 2406 | 2407 | 2408 | 2409 | 2410 | 2411 | 2412 | 2413 | 2414 | 2415 | 2416 | 2417 | 2418 | 2419 | 2420 | 2421 | 2422 | 2 |
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163. The synthase of claim 107, wherein said synthase has 50% or greater sequence identity to residues 579 to 847 of SEQ ID 44.
164. The synthase of claim 107, wherein said synthase has 60% or greater sequence identity to residues 579 to 847 of SEQ ID 44.
165. The synthase of claim 107, wherein said synthase catalyses the formation of a terpenoid product from a monoterpene substrate.
166. The synthase of claim 107, wherein said synthase catalyses the formation of a terpenoid product from a sesquiterpene substrate.
167. The synthase of claim 107, wherein said synthase catalyses the formation of a terpenoid product from a diterpene substrate.
168. The synthase of claim 165, wherein said product is a cyclic terpenoid hydrocarbon.
169. The synthase of claim 166, wherein said product is a cyclic terpenoid hydrocarbon.
170. The synthase of claim 167, wherein said product is a cyclic terpenoid hydrocarbon.
171. The synthase of claim 165, wherein said product is an acyclic terpenoid hydrocarbon.
172. The synthase of claim 166, wherein said product is an acyclic terpenoid hydrocarbon.
173. The synthase of claim 167, wherein said product is an acyclic terpenoid hydrocarbon.
174. The synthase of claim 165, wherein said product is a cyclic hydroxylated terpenoid hydrocarbon.
175. The synthase of claim 166, wherein said product is a cyclic hydroxylated terpenoid hydrocarbon.
176. The synthase of claim 167, wherein said product is a cyclic hydroxylated terpenoid hydrocarbon.

178. The synthase of claim 166, wherein said product is an acyclic hydroxylated terpenoid hydrocarbon.

180. The synthase of claim 107, wherein said amino acid at position 587 is selected from the group consisting of Cys, Ser, and Thr.

182. The synthase of claim 107, wherein said amino acid at position 587 is selected from the group consisting of Pro, Gly, and Ala.

184. The synthase of claim 107, wherein said amino acid at position 587 is selected from the group consisting of Met, Ile, Val and Leu.

186. The synthase of claim 107, wherein said amino acid at position 587 is selected from the group consisting of Gln, Asn and His.

188. The synthase of claim 107, wherein said amino acid at position 606 is selected from the group consisting of Phe, Tyr and Trp.

190. The synthase of claim 107, wherein said amino acid at position 606 is selected from the group consisting of Glu and Asp.

191. The synthase of claim 107, wherein said amino acid at position 606 is selected from the group consisting of Met, Ile, Val and Leu.

192. The synthase of claim 107, wherein said amino acid at position 606 is selected from the group consisting of Arg and Lys.

193. The synthase of claim 107, wherein said amino acid at position 606 is selected from the group consisting of Gln, Asn and His.

194. The synthase of claim 107, wherein said amino acid at position 714 is selected from the group consisting of Cys, Ser and Thr.

195. The synthase of claim 107, wherein said amino acid at position 714 is selected from the group consisting of Phe, Tyr and Trp.

196. The synthase of claim 107, wherein said amino acid at position 714 is selected from the group consisting of Pro, Gly, and Ala.

197. The synthase of claim 107, wherein said amino acid at position 714 is selected from the group consisting of Glu and Asp.

198. The synthase of claim 107, wherein said amino acid at position 714 is selected from the group consisting of Met, Ile, Val and Leu.

199. The synthase of claim 107, wherein said amino acid at position 714 is selected from the group consisting of Arg and Lys.

200. The synthase of claim 107, wherein said amino acid at position 714 is selected from the group consisting of Gln, Asn and His.

201. The synthase of claim 107, wherein said amino acid at position 715 is selected from the group consisting of Cys, Ser and Thr.

202. The synthase of claim 107, wherein said amino acid at position 715 is selected from the group consisting of Phe, Tyr and Trp.

203. The synthase of claim 107, wherein said amino acid at position 715 is selected from the group consisting of Pro, Gly, and Ala.

204. The synthase of claim 107, wherein said amino acid at position 715 is selected from the group consisting of Glu and Asp.

205. The synthase of claim 107, wherein said amino acid at position 715 is selected from the group consisting of Met, Ile, Val and Leu.

206. The synthase of claim 107, wherein said amino acid at position 715 is selected from the group consisting of Arg and Lys.

207. The synthase of claim 107, wherein said amino acid at position 715 is selected from the group consisting of Gln, Asn and His.

208. The synthase of claim 107, wherein said amino acid at position 716 is selected from the group consisting of Cys, Ser and Thr.

209. The synthase of claim 107, wherein said amino acid at position 716 is selected from the group consisting of Phe, Tyr and Trp.

210. The synthase of claim 107, wherein said amino acid at position 716 is selected from the group consisting of Pro, Gly, and Ala.

211. The synthase of claim 107, wherein said amino acid at position 716 is selected from the group consisting of Glu and Asp.

212. The synthase of claim 107, wherein said amino acid at position 716 is selected from the group consisting of Met, Ile, Val and Leu.

213. The synthase of claim 107, wherein said amino acid at position 716 is selected from the group consisting of Arg and Lys.

214. The synthase of claim 107, wherein said amino acid at position 716 is selected from the group consisting of Gln, Asn and His.

215. The synthase of claim 107, wherein said amino acid at position 719 is selected from the group consisting of Cys, Ser and Thr.

216. The synthase of claim 107, wherein said amino acid at position 719 is selected from the group consisting of Phe, Tyr and Trp.

217. The synthase of claim 107, wherein said amino acid at position 719 is selected from the group consisting of Pro, Gly, and Ala.

218. The synthase of claim 107, wherein said amino acid at position 719 is selected from the group consisting of Glu and Asp.

219. The synthase of claim 107, wherein said amino acid at position 719 is selected from the group consisting of Met, Ile, Val and Leu.

220. The synthase of claim 107, wherein said amino acid at position 719 is selected from the group consisting of Arg and Lys.

221. The synthase of claim 107, wherein said amino acid at position 719 is selected from the group consisting of Gln, Asn and His.

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222. The synthase of claim 107, wherein said amino acid at position 753 is selected from the group consisting of Cys, Ser and Thr.

223. The synthase of claim 107, wherein said amino acid at position 753 is selected from the group consisting of Phe, Tyr and Trp.

224. The synthase of claim 107, wherein said amino acid at position 753 is selected from the group consisting of Pro, Gly, and Ala.

225. The synthase of claim 107, wherein said amino acid at position 753 is selected from the group consisting of Glu and Asp.

226. The synthase of claim 107, wherein said amino acid at position 753 is selected from the group consisting of Met, Ile, Val and Leu.

227. The synthase of claim 107, wherein said amino acid at position 753 is selected from the group consisting of Arg and Lys.

228. The synthase of claim 107, wherein said amino acid at position 753 is selected from the group consisting of Gln, Asn and His.

229. The synthase of claim 107, wherein said amino acid at position 834 is selected from the group consisting of Cys, Ser and Thr.

230. The synthase of claim 107, wherein said amino acid at position 834 is selected from the group consisting of Phe, Tyr and Trp.

231. The synthase of claim 107, wherein said amino acid at position 834 is selected from the group consisting of Pro, Gly, and Ala.

232. The synthase of claim 107, wherein said amino acid at position 834 is selected from the group consisting of Glu and Asp.

233. The synthase of claim 107, wherein said amino acid at position 834 is selected from the group consisting of Met, Ile, Val and Leu.

234. The synthase of claim 107, wherein said amino acid at position 834 is selected from the group consisting of Arg and Lys.

235. The synthase of claim 107, wherein said amino acid at position 834 is selected from the group consisting of Gln, Asn and His.

236. The synthase of claim 107, wherein said amino acid at position 835 is selected from the group consisting of Cys, Ser and Thr.

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237. The synthase of claim 107, wherein said amino acid at position 835 is selected from the group consisting of Phe, Tyr and Trp.

238. The synthase of claim 107, wherein said amino acid at position 835 is selected from the group consisting of Pro, Gly, and Ala.

239. The synthase of claim 107, wherein said amino acid at position 835 is selected from the group consisting of Glu and Asp.

240. The synthase of claim 107, wherein said amino acid at position 835 is selected from the group consisting of Met, Ile, Val and Leu.

241. The synthase of claim 107, wherein said amino acid at position 835 is selected from the group consisting of Arg and Lys.

242. The synthase of claim 107, wherein said amino acid at position 835 is selected from the group consisting of Gln, Asn and His.

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### REMARKS

Applicants respectfully request entry of the amendments and remarks submitted herein. Claims 1-106 and 108-162 have been canceled. New claims 163-242 have been added. Support for new claims 163-242 can be found in the originally filed claims and throughout the specification. Accordingly, claims 107 and 163-242 are currently pending. Attached is a marked-up version of the changes being made by the current amendments. Applicants note that claim 107 correspond to Group 11 in the May 4, 2001 Restriction Requirement issued by the Examiner in the parent case. Consideration of the pending application is respectfully requested.

#### The Sequence Listing

Applicants submit herewith a paper copy of the Sequence Listing and a request to transfer the computer readable form of the Sequence Listing from the parent application to the present application. The Sequence Listing submitted herewith to replace the originally-filed Sequence Listing serves to conform SEQ ID NO:1 and SEQ ID NO:2 to the sequences of GenBank Accession Number Q40577 referred to on page 14 of the specification. The tobacco epiaristolochene synthase (TEAS) nucleic acid and protein sequences in SEQ ID NO:1 and 2 of the original Sequence Listing are those of Accession No. L04680. L04680 differs from the sequence of Q40577 at 22 nucleotides, resulting in differences at 6 amino acid positions (residues 44, 55, 62, 73, 89 and 388). The annotation for GenBank Accession No. Q40577 notes the differences between Q40577 and L04680. The text of the Q40577 GenBank annotations is enclosed. The differences between the two TEAS sequences do not correspond to any of the nineteen residues of TEAS referred to throughout the specification (*i.e.*, residues 270, 273, 294, 297, 298, 376, 401, 402, 403, 404, 407, 440, 444, 516, 519, 520, 525, 527, and 528). Accordingly, the sequence amendments to the Sequence Listing submitted herewith do not constitute new matter.

The Sequence Listing submitted herewith also clarifies or corrects several identifier fields within the originally-filed Sequence Listing. For example, SEQ ID NOS:1-12 originally used the mutant designation in the identifier field for organism. SEQ ID NOS:1-12 now identify *Nicotiana tabacum* as the organism. By way of this amendment, Applicants have also included the description for the 'n' nucleotides in SEQ ID NO:11 and the 'Xaa' residues in SEQ ID NO:12 that were absent in the originally-filed Sequence Listing. Applicants have also removed

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the stop codon from the polypeptide sequences of the Sequence Listing, as required under 37 CFR §1.821-1.825. The herein-described corrections to the Sequence Listing do not constitute an introduction of new matter.

CONCLUSION

Applicant asks that claim 107 and 163-242 be examined. The filing fee enclosed herewith reflects the claim amendments herein. Please apply any other charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: June 28, 2001

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

A paragraph concerning related applications was inserted on page 1 of the application after the title.

The Sequence Listing filed with the application was replaced with the Sequence Listing enclosed herein.

In the Claims:

Claims 1-106 and 108-162 have been cancelled.

The following new claims were added:

163. The synthase of claim 107, wherein said synthase has 50% or greater sequence identity to residues 579 to 847 of SEQ ID 44.

164. The synthase of claim 107, wherein said synthase has 60% or greater sequence identity to residues 579 to 847 of SEQ ID 44.

165. The synthase of claim 107, wherein said synthase catalyses the formation of a terpenoid product from a monoterpene substrate.

166. The synthase of claim 107, wherein said synthase catalyses the formation of a terpenoid product from a sesquiterpene substrate.

167. The synthase of claim 107, wherein said synthase catalyses the formation of a terpenoid product from a diterpene substrate.

168. The synthase of claim 165, wherein said product is a cyclic terpenoid hydrocarbon.

169. The synthase of claim 166, wherein said product is a cyclic terpenoid hydrocarbon.

170. The synthase of claim 167, wherein said product is a cyclic terpenoid hydrocarbon.

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172. The synthase of claim 166, wherein said product is an acyclic terpenoid hydrocarbon.

173. The synthase of claim 167, wherein said product is an acyclic terpenoid hydrocarbon.

174. The synthase of claim 165, wherein said product is a cyclic hydroxylated terpenoid hydrocarbon.

175. The synthase of claim 166, wherein said product is a cyclic hydroxylated terpenoid hydrocarbon.

176. The synthase of claim 167, wherein said product is a cyclic hydroxylated terpenoid hydrocarbon.

177. The synthase of claim 165, wherein said product is an acyclic hydroxylated terpenoid hydrocarbon.

178. The synthase of claim 166, wherein said product is an acyclic hydroxylated terpenoid hydrocarbon.

179. The synthase of claim 167 wherein said product is an acyclic hydroxylated terpenoid hydrocarbon.

180. The synthase of claim 107, wherein said amino acid at position 587 is selected from the group consisting of Cys, Ser, and Thr.

181. The synthase of claim 107, wherein said amino acid at position 587 is selected from the group consisting of Phe, Tyr and Trp.

182. The synthase of claim 107, wherein said amino acid at position 587 is selected from the group consisting of Pro, Gly, and Ala.

183. The synthase of claim 107, wherein said amino acid at position 587 is selected from the group consisting of Glu and Asp.

184. The synthase of claim 107, wherein said amino acid at position 587 is selected from the group consisting of Met, Ile, Val and Leu.

185. The synthase of claim 107, wherein said amino acid at position 587 is selected from the group consisting of Arg and Lys.

186. The synthase of claim 107, wherein said amino acid at position 587 is selected from the group consisting of Gln, Asn and His.

187. The synthase of claim 107, wherein said amino acid at position 606 is selected from the group consisting of Cys, Ser and Thr.

188. The synthase of claim 107, wherein said amino acid at position 606 is selected from the group consisting of Phe, Tyr and Trp.

189. The synthase of claim 107, wherein said amino acid at position 606 is selected from the group consisting of Pro, Gly, and Ala.

190. The synthase of claim 107, wherein said amino acid at position 606 is selected from the group consisting of Glu and Asp.

191. The synthase of claim 107, wherein said amino acid at position 606 is selected from the group consisting of Met, Ile, Val and Leu.

192. The synthase of claim 107, wherein said amino acid at position 606 is selected from the group consisting of Arg and Lys.

193. The synthase of claim 107, wherein said amino acid at position 606 is selected from the group consisting of Gln, Asn and His.

194. The synthase of claim 107, wherein said amino acid at position 714 is selected from the group consisting of Cys, Ser and Thr.

195. The synthase of claim 107, wherein said amino acid at position 714 is selected from the group consisting of Phe, Tyr and Trp.

196. The synthase of claim 107, wherein said amino acid at position 714 is selected from the group consisting of Pro, Gly, and Ala.

197. The synthase of claim 107, wherein said amino acid at position 714 is selected from the group consisting of Glu and Asp.

198. The synthase of claim 107, wherein said amino acid at position 714 is selected from the group consisting of Met, Ile, Val and Leu.

199. The synthase of claim 107, wherein said amino acid at position 714 is selected from the group consisting of Arg and Lys.

200. The synthase of claim 107, wherein said amino acid at position 714 is selected from the group consisting of Gln, Asn and His.

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215. The synthase of claim 107, wherein said amino acid at position 719 is selected from the group consisting of Cys, Ser and Thr.

230. The synthase of claim 107, wherein said amino acid at position 834 is selected from the group consisting of Phe, Tyr and Trp.



231. The synthase of claim 107, wherein said amino acid at position 834 is selected from the group consisting of Pro, Gly, and Ala.

232. The synthase of claim 107, wherein said amino acid at position 834 is selected from the group consisting of Glu and Asp.

233. The synthase of claim 107, wherein said amino acid at position 834 is selected from the group consisting of Met, Ile, Val and Leu.

234. The synthase of claim 107, wherein said amino acid at position 834 is selected from the group consisting of Arg and Lys.

235. The synthase of claim 107, wherein said amino acid at position 834 is selected from the group consisting of Gln, Asn and His.

236. The synthase of claim 107, wherein said amino acid at position 835 is selected from the group consisting of Cys, Ser and Thr.

237. The synthase of claim 107, wherein said amino acid at position 835 is selected from the group consisting of Phe, Tyr and Trp.

238. The synthase of claim 107, wherein said amino acid at position 835 is selected from the group consisting of Pro, Gly, and Ala.

239. The synthase of claim 107, wherein said amino acid at position 835 is selected from the group consisting of Glu and Asp.

240. The synthase of claim 107, wherein said amino acid at position 835 is selected from the group consisting of Met, Ile, Val and Leu.

241. The synthase of claim 107, wherein said amino acid at position 835 is selected from the group consisting of Arg and Lys.

242. The synthase of claim 107, wherein said amino acid at position 835 is selected from the group consisting of Gln, Asn and His.

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